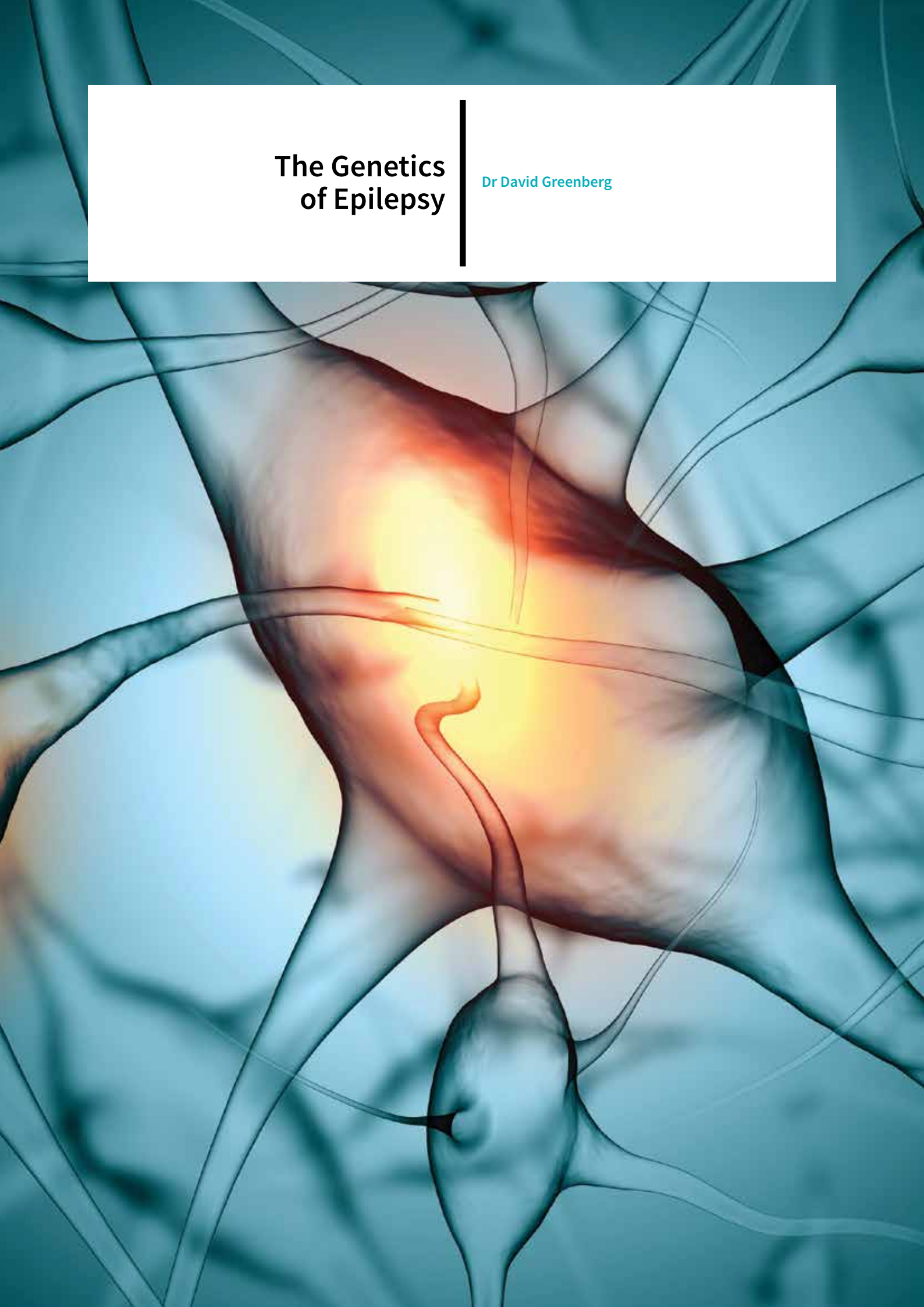
The Genetics of Epilepsy

Dr David Greenberg



THE GENETICS OF EPILEPSY

Searching for disease-causing genes is no simple task. **Dr David Greenberg** has been studying the genetic determinants of inherited epilepsy for 30 years and explains how the search can be hampered by deeply ingrained, but incorrect, assumptions within the field

Epilepsy is thought to affect around 1% of the worldwide population. It is defined as a neurological disease, or group of diseases, causing epileptic seizures. The seizures can have a broad range of symptoms, severity, and duration, depending on the specific type of epilepsy. Seizures can be very brief and practically undetectable but in more severe cases can cause prolonged and vigorous episodes of shaking and/or loss of awareness. Seizures are a manifestation of a wave of unregulated electrical activity in the brain.

One of the most useful tools on the route to developing treatments for any disease is a good knowledge of its causes. We have known for a long time that many types of epilepsy are heritable diseases. Some very rare forms of epilepsy can be seen in one generation after the other in a family, but such generation-to-generation manifestation of epilepsy is not the common pattern. The most common forms of the epilepsies with a strong genetic effect are the idiopathic generalised epilepsies (IGEs) (also called Genetic Generalized Epilepsies (GGE)), which constitute around a third of all cases of epilepsy and include various sub-types, such as juvenile myoclonic epilepsy (JME) and juvenile absence epilepsy (JAE), among other forms. The familial nature of these syndromes makes it likely that the basic causes are genetic, that is, they are caused by mutations to genes and are not caused by anything environmental. However, not only have the genes involved proven difficult to identify, there is virtually no knowledge of what has gone wrong in brain wiring or function that leads to these epilepsies. Therefore, if the mutated gene, or genes, can be identified, we will be well on the way to understanding the causes of the IGEs, allowing researchers to have a target for developing better treatments and even cures. Unfortunately, the reality of this research is not quite so simple. Dr David Greenberg has been studying the genetic causes of epilepsy for over 30 years and in addition to his own research on the subject, has written articles describing some of the core issues in the way geneticists search for disease causing genes.

Improving the search with careful diagnosis

Modern scientific technology allows researchers looking for the genetic causes of disease to generate large quantities of data more quickly than ever before. When Dr Greenberg began his research into this area, sequencing a gene could take years. Today this task can be performed in just a few hours, and entire genomes are sequenced with relative ease. Generating the data is no longer the hurdle it used to be. However, the technology does not help in what is perhaps the most important part of studying the genetic origins of epilepsy, namely, finding and choosing the human subjects for study, for the utmost care must be exercised in selection of subjects. One of Dr Greenberg's main criticisms with the way research is carried out in this area concerns this selection, specifically at the very beginning of the process: the diagnosis of epilepsy. As previously mentioned, 'epilepsy' is really a group of diseases, producing a wide spectrum of symptoms consisting of different types of seizures, different timings, different seizure triggers, different ages at which the seizures start, and different treatments. There is little evidence to suggest that just because these symptoms are all called 'seizures', that they are all produced by perturbing the function of any particular gene, or group of genes. In order to find the genes associated with a particular type of IGE, a detailed system of disease classification is first required.

Psychiatry as a field is often criticised for making an ever expanding list of diseases with more and more specific symptoms. Where once a person may have been diagnosed with just 'anxiety', there is now a vast range of specific disorders that contain 'anxiety' as a symptom. After receiving criticism for dividing so many disorders into various subcategories, a trend developed in psychiatry to try to simplify the field by lumping many of these disorders back into single categories. One example of this is the recent decision to do away with the specific diagnoses of Asperger's syndrome, pervasive developmental delay (PDD) and autism, and, instead, to replace these definitions with the blanket term of 'autism spectrum disorder'. Though there may be some organisational benefits to this type of approach, it causes problems when trying to find specific genetic factors that determine the expression of diseases such as epilepsy because 'epilepsy' is a symptom (recurrent seizures) not a single disease. What we call epilepsy, even IGE, is a heterogeneous group of diseases that are difficult to differentiate without a carefully considered diagnosis.

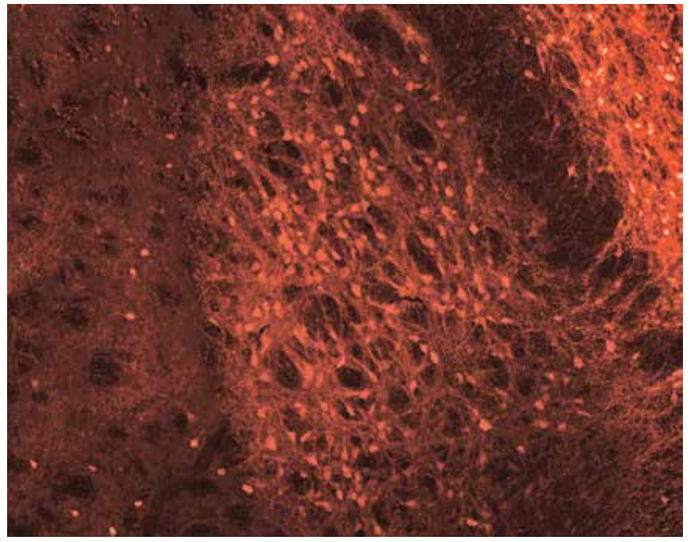
That careful diagnosis, which is based on accurate descriptions of known types of epilepsy, is critical because, without it, genetic analysis becomes incredibly difficult. Sufferers who display similar, but not identical, symptoms may be categorised as having the same disease, but these disorders may be due to mutations in completely different genes or different combinations of genes. Using genetic data from hundreds of people to look for mutations associated with a particular disease becomes an uphill battle if the patients have a wide range of different diseases, and therefore different causes. This is the problem of genetic heterogeneity, perhaps the biggest problem in trying to understand epilepsy as well as other common diseases.

The accepted dogma of the causes of epilepsy

Dr Greenberg's critique of much of the research into the genetic determinants of epilepsy extends to some of the finer points of statistics and genetic analysis, but often what he takes issue with is the actual logic of how experiments are designed and the assumptions that can dictate how data are interpreted. A pervasive idea is that genetic diseases are often 'caused' by a single particular gene. This idea oversimplifies the reality of disease genetics. Although life would be much easier for researchers if diseases were usually caused by a single gene, the truth appears to be that often, multiple mutations (or variations) are required for a particular disease to rear its head (this is also known as gene-gene interaction). Rather than looking for rare versions of genes which are quite uncommon in epilepsy sufferers, Dr Greenberg suggests that we should also be looking at the pairs (or more) of common genes which only appear together in IGE sufferers. Mathematically, this makes sense, but means that when looking for a gene which causes a disease affecting only 1% of people, you might now be looking for a gene mutation (or variant) that can be found in as much as 10% or even 25% of the population but that 10% or 25% do not have the other gene (or genes) necessary to have the disease. Despite the high prevalence of these genes in the population, the disease will only manifest itself when both genes are inherited at the same time in the same person, in agreement with the observed frequency of epilepsy sufferers.

Dr Greenberg also warns his fellow scientists against susceptibility to 'blinders' in their study of epilepsy genetics. For many years, the field's most widely accepted idea was that epilepsy is a 'channelopathy', in that it is caused by problems in the proteins that channel the flow of ions across cell membranes. That flow of ions is the basis of electrical signals transmitted through the nervous system. This idea fits with observations of what happens to the brain during an epileptic seizure (a dysregulation of that ion flow), but the idea that most epilepsy is due to such 'broken' proteins is not supported by actual evidence. Dr Greenberg argues that this dogma caused researchers to be blinkered and adopt a biased interpretation of their data. During a genetic screen to look for mutations associated with epilepsy, where genetic data has been collected from a large number of people with and without epilepsy, the screen will first narrow down the search to a particular region of the genome. This region might contain a large number of genes, but researchers often became fixated only on those regions that happened to contain channel genes, which they assumed were the gene causing the epilepsy, focussing on these at the expense of any proper investigation of any of the other genes. Harmless variations exist in all our genes (those variations are what make us different from one another). If a family being studied has one of the benign variations in a gene pre-accepted as a cause

'We still understand very, very little about how the genome works. We have the letters of the book, but having the letters does not mean we understand the words'



The glowing neurons have been stained for the parvalbumin molecule. These special neurons, which express the neurotransmitter GABA (gamma-aminobutyric acid), are critical to controlling electrical activity in the brain

epilepsy (e.g., a channel gene), then we can end up with the kind of circular reasoning which leads to the 'channelopathy' hypothesis supporting itself without a leg to stand on. The presence of a mutation within the channel gene (or any other), also might not tell us much without the knowledge of how often mutations (or variations) are acquired by that gene in the general population over the course of time, which is a question often left unaddressed.

Our current understanding

Some genes have been identified thus far in the study of epilepsy genetics but most of the proven genes are for the rarest, most devestating forms of epilepsy. The first gene for an IGE (in fact, the first epilepsy-related gene discovered), was BRD2, and it was reported by Dr Greenberg's research group in 1988. BRD2, found on human chromosome 6, is associated specifically with Juvenile Myoclonic Epilepsy (JME), one of the most common forms of IGE. 'By first doggedly pursing and then proving our original finding of a gene on chromosome 6, we could then develop a mouse model that we have shown has symptoms very close to what we see in human JME', Dr Greenberg tells *Scientia*. 'This gives us a way to study what the gene is actually doing'.

What became clear is that BRD2 is not a channel gene, and instead belongs to a class of genes known as transcription factors. These genes exert control over the way other genes are expressed , or 'read' (i.e., copied onto RNA), and ultimately translated into proteins, depending on the type of cell and the requirements of that particular cell. When the function of the gene is knocked out entirely, the mice fail to develop a functional nervous system during gestation and do not survive, showing that the gene is required for normal brain development. However, when its function is reduced by half, as in the genetically modified mice, they have less of a particular type of neuron in parts of the brain, and they develop sensitivity to seizures on the same 'schedule' (i.e., after puberty) just as is seen in humans.

The specific location of the BRD2 mutation associated with JME is located within a 'non-coding' part of the gene, the part that does not directly determine the sequence of amino-acids making up the BRD2 protein. In the early days of gathering sequence data, geneticists believed non-coding DNA, which makes up 98% of the human genome, to be 'junk'. Sequencing of the coding regions only makes the task of sequencing much quicker and easier, but as the case of BRD2 shows us, it is not safe to assume that all disease-related mutations will be located in the part of the gene that becomes protein, or that parts of the genome without a clear function are 'junk'.

Another gene identified in a similar manner, and with some confidence as having a part to play in susceptibility to an epilepsy, is ELP4, which is associated with a common form of epilepsy called rolandic epilepsy (RE). Again, ELP4 is not a channel gene, and the presumptive causative mutation was not located within the coding region of the gene. Similar to BRD2, ELP4 may have a role in the development of the nervous system. ELP4 has a role in the construction of cellular structures called microtubules, part of the cell's 'cytoskeleton'. Microtubules are critical for the correct structural organisation of cells and the tissues which they are part of.

Both of these groups of studies employed a type of genetic analysis called 'linkage analysis'. Unlike an association analysis, where genetic markers (or signposts) in a large group of people with a particular disease are compared to the markers in people who do not have the

disease, a linkage analysis uses data collected from entire families. The benefit of this analysis is that it provides information about inheritance, which association analysis does not. One can see more clearly which genes cause disease when, for example, an affected parent carries a particular mutation and has several children, but only the ones who inherited the mutation also have from the disease. A disadvantage to this approach is that it is less sensitive for identifying genes with a more minor effect on the expression of the disease.

Another way of strengthening this analysis for identifying IGE genes, is, in addition to counting as 'affected', those with an actual epilepsy diagnosis, also counting those with epilepsy-associated brain activity by performing electroencephalograms (EEG) on the family members, even if those subjects do not have seizures. IGE patients usually have abnormal EEG findings, even when they are not having a seizure. Family members in families identified through and IGE patient have a higher frequency of these same EEG traits, even though they may never have had a seizure. In a particular family with a known history of epilepsy, after careful analysis of their genetics, we might see that the same genes are linked to the EEG trait as well as the epilepsy. When combined with the EEG data, the causative genes can be more readily identifiable by looking at who really displayed the neurological characteristics of the IGE, rather than just counting who suffers from epileptic seizures. This technique was used effectively in the identification of both ELP4 and BRD2.

The conclusion appears to be that rather than mutation within the channel genes being the root of the problem, many epilepsies may be caused instead by subtle anomalies in brain structure, which can cause or predispose a person to epileptic seizures.

The next steps

Dr Greenberg's research group plans to continue researching BRD2 to determine the mechanism by which it contributes to epilepsy susceptibility. They also aim to investigate what other genes BRD2 might interact with in people with epilepsy. As the root causes of the disease become more clear, so too will potential treatments. On the subject of increased knowledge of the causes of epilepsies and affected types of brain cells, Dr Greenberg says optimistically, 'not only can this lead to better treatments, but also even a cure for epilepsy'.

Meet the researcher

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Dr David Greenberg began his career in theoretical chemistry before changing fields to neurobiology and then on to human genetics. Working at the Harbor Hospital in Los Angeles, he researched the genetic causes of celiac disease and type 1 diabetes. Following this work, he moved on to the Comprehensive Epilepsy Centre at UCLA, to begin his research on the genetics of epilepsy. He now works at the Battelle Centre for Mathematical Medicine at the Nationwide Children's Hospital, Columbus, Ohio. Dr Greenberg has also developed computer software for conducting genetic analysis simulations. He has published extensively on these and developed a suite of programs used to teach linkage analysis and association analysis to researchers and students.

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